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Actin and Actin-binding Proteins II

2867-Pos Board B559
Structural Analysis of Intrinsically Disordered Protein (IDP): TRIOBP
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TRIOBP is an actin-bundling protein. Mutations of TRIOBP are associated with human deafness DFNB28. TRIOBP has three isoforms, named TRIOBP-1, TRIOBP-4, and TRIOBP-5. In vitro, TRIOBP isoform 4 (TRIOBP-4) forms dense F-actin bundles resembling the inner ear hair cell rootlet structure. Deletion of TRIOBP isoforms 4 and 5 leads to hearing loss in mice due to the absence of stereocilia rootlets. The mechanism of actin bundle formation by TRIOBP is not fully understood. The amino acid sequences of TRIOBP isoforms 4 and 5 contain two repeated motifs, referred to here as R1 and R2. Recent our study demonstrated that R1 motif is the major actin-binding domain of TRIOBP-4, and the binding of R2 motif with actin filaments is nonspecific. Structural analysis of TRIOBP by amino acid sequence showed ID proteins. Thus the second structure of TRIOBP may not have. To investigate the structural property of TRIOBP-4, we analyzed the structure of TRIOBP-4 by using circular dichroism, dynamic light scattering, and fluorescence correlation spectroscopy. Our analysis show that TRIOBP has a beta-sheet, but not alpha-helix. To investigate the structure of tight F-actin bundle structure with TRIOBP, we analyzed 3-D structure of the bundle by using 3-D image analysis from transmitted electron microscope images.

2868-Pos Board B560
Myosin II Does it All: Assembly, Remodeling, and Disassembly of Actin Networks are Governed by Myosin II Activity
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Eukaryotic cells rely on their cytoskeleton to carry out coordinated motion. To adapt to the changing requirements, the cell's cytoskeleton constantly remodels through the action of myosin II motors that interact with numerous actin filaments simultaneously. Here we study the various roles of myosin II clusters in the formation and evolution of *in-vitro* actomyosin networks. In our experiments the motor clusters can vary in size between 14 and 144 myosin II molecules and apply forces ranging between several to tens of pico Newtons. During the initial process of network formation the motor clusters become embedded within the network structure, where they act as internal active cross-linkers. Myosin II clusters enhance the nucleation of actin network in a concentration dependent manner, in the presence of passive crosslinkers, thus functioning as a '*network co-nucleator*'. As network formation is achieved, myosin II turns into a '*network reorganizer*', where it takes part in the remodeling and coarsening of the overall network structure. As a result of the strong confinement (the motor clusters within the network bundles exhibit high processivity with a fraction of attached motors $p_{att} \geq 0.15$), their effect in the nucleation and reorganization of the actin network is enhanced, rendering even small clusters of 14 myosin II molecules efficient. The stresses building-up in the networks lead to complex dynamics and can drive their contraction and rupture, depending on motor concentration and cluster size. Above a certain concentration, the severing and disassembly properties of the motors dominate, and they function as '*network disassembly agents*'. Myosin II motors are shown to be unique motors that function as complex machines that perform a diversity of tasks, thereby regulating the nature of the assembled network and facilitating its formation.

2869-Pos Board B561
Regulation of Actin Dynamics by Tropomyosin
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It is widely accepted that tropomyosin stabilizes actin filaments mechanically and also by protecting against the action of filament destabilizing proteins. The mechanisms underlying these effects are still unclear. To investigate the influence of tropomyosin on the actin filament severing activity of gelsolin we measured both the F-actin viscosity and the relative number concentrations of filaments after fragmentation by either gelsolin alone or by gelsolin/tropomyosin complexes. Our results show that the association of muscle tropomyosin with F-actin did not significantly protect the filaments from being severed by gelsolin. On the other hand, preceding interaction of gelsolin with tropomyosin reduced the severing activity of gelsolin by up to 80%. These results suggest that tropomyosin is involved in the modulation of actin dynamics by binding gelsolin in solution to prevent it from severing. We also show that in proteolytically modified F-actin where the turnover of subunits is strongly enhanced, tropomyosin restored the stability of this actin but did not stabilize it against the disruptive effects of centrifugal forces and shear stress. Instead of this, tropomyosin inhibited the steady-state ATP hydrolysis of proteolytically modified actin in a cooperative manner, with half-maximal and maximal effects observed at TM:actin molar ratios of about 1:50 and 1:8, respectively. Thus, stabilization of actin filaments by tropomyosin involves conformational changes which seem to modify the monomer-monomer contacts along the filament. We believe that both interaction with gelsolin and stabilization of intermonomer contacts within F-actin may contribute to the regulation by tropomyosin of cytoskeleton dynamics.

2870-Pos Board B562
Regulation of Nonmuscle Myosin II by Tropomyosin Isoforms
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Tropomyosin (Tm) regulates actin myosin interactions in eukaryotic cells ranging from yeast to mammalian muscle and nonmuscle cells. Tropomyosin is a α-helical coiled-coil actin binding protein that associates end-to-end to form continuous strands along both sides of the actin filament. Tropomyosins can inhibit or activate actomyosin MgATPase activity and motility depending on the myosin and Tm isoforms. In this study, we have attempted to determine whether activation or inhibition is specified by the Tm or the myosin isoform. We carried out in vitro motility assays with four myosin isoforms (skeletal muscle myosin, and nonmuscle IIA, IIB and IIC HMMs) in the presence of five Tm isoforms (skeletal muscle αTm, and nonmuscle isoforms, Tm2, Tm5a, Tm5NM1 and Tm4) and skeletal muscle actin. With skeletal muscle myosin, actin-Tm filament velocities were inhibited by αTm (~60%) but activated by Tm5a and Tm5NM1 (30-60%) relative to actin alone, whereas Tm2 and Tm4 had little or no effect. In the case of nonmuscle IIA and IIC HMMs, all nonmuscle Tms activated filament velocities, whereas αTm had no effect, relative to actin alone. None of the Tm isoforms affected filament velocities with nonmuscle IIB HMM. Therefore, the primary determinant of the effect of Tm on actin filament velocities on myosin is the myosin isoform. The actin-activated MgATPase activities of IIA, IIB and IIC HMMs were measured to determine the mechanism of activation by a nonmuscle Tm, Tm5NM1. Tm5NM1 increased the V_{max} of both IIA (26%), and IIC (19%), with a much smaller effect on IIB (12%) compared to actin alone thus supporting the motility data. Tm5NM1 also decreased the K_{ATPase} of IIA and IIB. Therefore, Tm5NM1 activates the MgATPase activity of IIA and IIB by increasing the V_{max} and decreasing K_{ATPase}. *Supported by NIH.*

2871-Pos Board B563
Geometrical and Mechanical Properties Control Actin Filament Organization
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The organization of actin filaments into higher-ordered structures governs eukaryotic cell shape and movement. For all these processes, the collective behavior of the actin filaments is governed by the environmental conditions (associated proteins, geometry, confinement...).We demonstrated recently that nucleation geometry governs ordered actin structure organization (Reymann et al., 2010) but the mechanisms behind this control were not clearly established. To understand how the geometrical parameters governed the actin dynamics, we simulated the filament growth from pattern with the cytoskeleton simulation software Cytosim. The simulation parameters were first calibrated by matching in-vitro and simulated filaments behavior from a simple pattern. The steric interaction between filaments was particularly crucial to obtain a good match between experimental and simulated actin architecture.
We then used the simulations to observe the effect of the pattern geometry and filament rigidity on the overall organization of the actin structures. We observed that both the nucleation geometry and the mechanical properties of actin filaments are essential to built from a common pool of actin monomers the diversity of actin organizations observed in vivo (parallel or antiparallel bundles and actin networks). Then we studied the confined behavior of growing filaments, and showed how the relative rigidity of the filament (compared to the confinement size) affects its bending ability and its growing speed. By comparing simulations and experimental results, we determined how